not include specific descriptions of combinations of IL-15 with chemokines such as CCL19.

[0012]

Non Patent Literature 6 discloses a CAR-T cell expressing IL-15 and/or IL-21 and targeting GPC3. However, Non Patent Literature 6 does not include specific descriptions of combinations of the specific cytokines with chemokines such as CCL19.

Citation List

Patent Literature

[0013]

Patent Literature 1: WO 2016/056228

Patent Literature 2: WO 2007/037780

Patent Literature 3: WO 2013/040371

Patent Literature 4: WO 2014/186469

Patent Literature 5: US 2013/0071414

Non Patent Literature

[0014]

Non Patent Literature 1: Adachi et al., Nature Biotechnology, VOL 36, No 4, 346-353

Non Patent Literature 2: Hurton et al., Proc Natl Acad Sci., 113 (48), E7788-97, 2016

Non Patent Literature 3: Mortier et al., J Biol Chem. 281

Those skilled in the art would be able to appropriately convert the embodiment of the T cell of the present invention to be obtained by allowing the T cell to express the predetermined cytokine and chemokine together with a CAR, and relevant embodiments thereto such as the medicament of the present invention, the expression vector of the present invention, and the production method of the present invention into an embodiment of immune cells other than T cells (such as NK cells, monocytes, macrophages, and dendritic cells) expressing the predetermined cytokine and chemokine together with a CAR and relevant embodiments thereto such as a medicament, an expression vector, and a production method, in consideration of the contents disclosed herein as a whole.

Advantageous Effects of Invention

[0023]

The persistence and proliferation of the T cell of the present invention are improved by the T cell expressing the predetermined cytokine (such as IL-15) and chemokine (CCL19). Therefore, the antitumor activity by the CAR can be enhanced (e.g., reduction in the number of residual tumor cells, improvement in amount of IFNγ to be produced, and improvement in migration and accumulation of host immune cells (such as T cells, dendritic cells, NK cells) in the tumor site).

Further, the therapeutic effect on cancer can be improved by a medicament containing the T cell of the present invention. In particular, the T cell of the present invention may be involved in NK cell activation and therefore may be useful for the treatment of carcinomas sensitive to NK cells (such as melanoma, Merkel cell cancer, colorectal cancer, kidney cancer, breast cancer, ovarian cancer, fallopian tube cancer, cervical cancer, liver cancer, lung cancer, non-small cell lung cancer, head and neck cancer, small intestine cancer, prostate cancer, bladder cancer, rectal cancer, pancreatic cancer, Ewing's sarcoma, rhabdomyosarcoma, nasopharyngeal cancer, esophageal cancer, biliary tract cancer, neuroblastoma, osteosarcoma, acute myeloid leukemia, multiple myeloma, lymphoma, and leukemia). Further, since the enhancement of the antitumor activity reduces the number of cells to be administered, effects such as reduction in side effects such as CRS (Cytokine Release Syndrome) and reduction in production cost are expected.

[0024]

The combination of the predetermined cytokine and chemokine of the present invention exerts action and effect that various CAR-expressing immune cells with high antitumor activity can be obtained even in the case where the genes are transferred and expressed together with a CAR gene in immune

aforementioned signaling domain (intracellular domain) can also be used. In one embodiment of the present invention, the signaling domain preferably contains three intracellular domains, CD28, 4-1BB, and CD3ζ.

[0051]

In the case where the signaling domain contains a plurality of intracellular domains, the intracellular domains may be linked to each other via linker peptides (oligopeptides or polypeptides) consisting of 2 to 10 amino acids. Examples of such linker peptides can include a peptide consisting of a glycine-serine contiguous sequence.

[0052]

Each between the single-chain antibody and the transmembrane domain, and between the transmembrane domain and the signaling domain, a spacer region which is a peptide (oligopeptide or polypeptide) consisting of any amino acid sequence, having a length of 1 to 100 amino acids, preferably 10 to 50 amino acids may be included. Examples of the spacer region can include a peptide consisting of a glycine-serine contiguous sequence.

[0053]

The amino acid sequence of the aforementioned CAR expressed by the T cell of the present invention may be appropriately set depending on the application of the T cell,

$_{sc}$IL27_F2A_CCL19 DNA fragment (DNA fragment #9), or in the amino acid sequences of a fusion protein containing SEQ ID NO: 5: IL15$_{LSP}$, a fusion protein containing SEQ ID NO: 7: $_{s}$IL15$_{RA}$, a fusion protein containing SEQ ID NO: 9: $_{mb}$IL15$_{RA}$, a fusion protein containing SEQ ID NO: 11: $_{sushi}$IL15, a fusion protein containing SEQ ID NO: 13: IL-18, a fusion protein containing SEQ ID NO: 15: IL-21, and a fusion protein containing SEQ ID NO: 17: $_{sc}$IL27.

[0070]

(3) Chemokine

The chemokine expressed by the T cell of the present invention is CCL19. CCL19 is mainly produced from dendritic cells and macrophages of lymph nodes and has a function of inducing migration of T cells, B cells, and mature dendritic cells via their receptor, CCR7. That is, "CCL19" in the present invention includes not only the full-length protein (polypeptide) consisting of the amino acid sequence of the natural CCL19, but also a part thereof (functional partial polypeptide) and variants of the whole or a part of the natural CCL19 in which one or a plurality of amino acid sequences are deleted, replaced or added to the natural amino acid sequence (preferably, within the range having the later-described homology).

[0071]

pentostatin, fludarabine, cladribine, methotrexate, 5-fluorouracil, 6-mercaptopurine, and enocitabin; molecular targeted drugs such as rituximab, cetuximab, and trastuzumab; kinase inhibitors such as imatinib, gefitinib, erlotinib, afatinib, dasatinib, sunitinib, and trametinib; proteasome inhibitors such as bortezomib; calcineurin inhibitors such as cyclosporine and tacrolimus; antitumor antibiotics such as idarubicin, doxorubicin and mitomycin C; plant alkaloids such as irinotecan and etoposide; platinum preparations such as cisplatin, oxaliplatin, and carboplatin; hormone therapeutic drugs such as tamoxifen and bicalutamide; and immunoregulatory drugs such as interferon, nivolumab, and pembrolizumab.

[0082]

Use of the medicament of the present invention in combination with additional anticancer agent may be in any form of (a) using the additional anticancer agent and then the medicament of the present invention, (b) using the medicament of the present invention and the additional anticancer agent at the same time, or (c) using the medicament of the present invention and then the additional anticancer agent. When the medicament of the present invention is used in combination with the additional anticancer agent, it is expected that the therapeutic effect

[0088]

The sugar-modified nucleotide analogs may be any analog obtained by adding any chemical structural material to or replacing with any chemical structural material part or the whole of the chemical structure of the sugar of the nucleotide. Specific examples thereof can include nucleotide analogs replaced with 2'-O-methyl ribose, nucleotide analogs replaced with 2'-O-propyl ribose, nucleotide analogs replaced with 2'-methoxyethoxy ribose, nucleotide analogs replaced with 2'-O-methoxyethyl ribose, nucleotide analogs replaced with 2'-O-[2-(guanidinium) ethyl] ribose, nucleotide analogs replaced with 2'-fluororibose, crosslinked artificial nucleic acids having two cyclic structures by introducing crosslinked structures into the sugar part (Bridged Nucleic Acids) (BNAs), more specifically, locked artificial nucleic acids in which the oxygen atom at the 2' position and the carbon atom at the 4' position are crosslinked via methylene (Locked Nucleic Acids) (LNAs), and ethylene crosslinked artificial nucleic acids (Ethylene bridged nucleic acids) (ENAs) [Nucleic Acid Research, 32, e175 (2004)], and further include peptide nucleic acids (PNAs) [Acc. Chem. Res., 32, 624 (1999)], oxypeptide nucleic acids (OPNAs) [J. Am. Chem. Soc., 123, 4653 (2001)], and peptide ribonucleic acids (PRNAs) [J. Am. Chem. Soc., 122, 6900 (2000)].

In the expression vector-introducing step, T cells may be cultured by bringing such a preparation into contact with T cells, generally, in a medium to which a preparation containing the expression vector.

[0105]

In a preferred embodiment of the present invention, the expression vector of the present invention is introduced into T cells by a virus infection method. For example, a method in which the vector of the present invention and the packaging vector (plasmid) of each virus are transfected into a host cell, using a commercially available kit corresponding to each viral vector such as a retroviral vector, a lentiviral vector, an adenoviral vector, and an adeno-associated viral vector, to produce a recombinant virus, and thereafter T cells are infected with the recombinant virus obtained can be mentioned. Examples of the commercially available kit for viral vectors include Retrovirus packaging Kit Eco (available from Takara Bio Inc). Examples of the host cell include GP2-293 cell (available from Takara Bio Inc.), Plat-GP cell (available from Cosmo Bio Co., Ltd.), PG13 cell (ATCC CRL-10686), and PA317 cell (ATCC CRL-9078).

[0106]

The method for producing a CAR-T cell of the present invention may further contain other steps before and after